REMARKS/ARGUMENTS

The following remarks and arguments are submitted in response to the Office Action mailed January 5, 2005. Claims 13-36 are pending in the application. Claims 13-26 stand rejected and claims 14-24 and 26-36 are objected to. Applicants have amended claims 13 and 25 in order to more particularly point out and distinctly claim the present invention. In view of the remarks and arguments presented below, and the amendments to the claims, Applicants believe that the basis of the rejections have been overcome and respectfully request reconsideration of the application and withdrawal of the outstanding rejections.

Summary of Claimed Invention

The present invention is directed to a kit for sequencing one or more DNA regions from a sample, consisting of, in packaged combination, a single reaction vessel for sequencing each DNA region, wherein each reaction vessel contains a mixture of region-specific sequencing reagents for obtaining bi-directional sequence of each DNA region of interest (i.e., the sense and anti-sense strand of each DNA region), and optionally one or more non-region specific sequencing reagents. The region-specific sequencing reagents comprise region-specific primers. The non-region specific sequencing reagents (which can readily be provided by the user using standard reagents, and are therefore optional components of the claimed kit) are selected from one or more of the group consisting of deoxynucleotide triphosphate feedstocks, at least one chain terminating dideoxynucleotide triphosphate and a thermally stable polymerase enzyme capable of incorporating dideoxynucleotides into an extending nucleic acid polymer.

Amendment of Claims

Claims 13 and 25 have been amended so as to more particularly point out and distinctly claim the present invention.

Consistent with the description of the invention in the specification, claim 13 has been amended to clarify that the kit of the present invention includes a single reaction vessel that is used "for each DNA region to be sequenced" and that each reaction vessel contains a mixture of region-specific sequencing reagents sufficient for "sequencing the sense and anti-sense strand of each DNA region to be sequenced." Support for this amendment is found in the specification at, for example, page 4, lines 1-2; page 5, lines 2 and 5; page 7, lines 21-26; and page 11, lines 9-10.

Claim 13 has further been amended to clarify that the deoxynucleotide triphosphate feedstocks, the chain terminating dideoxynucleotide triphosphates and the thermally stable polymerase enzyme capable of incorporating dideoxynucleotides into an extending nucleic acid polymer are "non-region specific sequencing reagents."

Claim 25 has been amended to recite the term "reaction vessel," which has antecedent basis to the term "reaction vessel" in claim 13, from which claim 25 depends.

Claim Rejections Under 35 USC §112

The Examiner has rejected claims 13-36 under 35 U.S.C. §112, as being indefinite for failing to particular point out and distinctly claiming the subject matter of the invention. The Examiner specifically states that claim 25 (which depends from claim 13) lacks proper antecedent basis for "plurality of tubes of region-specific reagents," and has suggested that amendment of claim 25 using language corresponding to claim 13 would overcome the rejection.

Applicants have accordingly amended claim 25 to recite a "plurality of reaction vessels," consistent with the language of claim 13. Antecedent basis for the phrase "plurality of reaction vessels" in claim 25 is found in claim 13, which recites a "reaction vessel for each DNA region to be sequenced." Applicants respectfully submit that claim 25 now has proper antecedent basis, and request that the rejection under §112 be withdrawn.

Claim Rejections Under 35 USC §103

The Examiner has also rejected claim 13 (as well as claims 14-36 which depend form claim 13) as being unpatentable over Mian et al. (US 5,683,657) in view of Ahern (The Scientist, Vol. 9, No. 14, pages 1-15, June 1995). The Examiner asserts that Mian et al. teaches "a method comprising the use of a mixture of region-specific reagents in a single reaction vessel, wherein said mixture comprises primer deoxynucleotide triphosphate feedstocks, at least one chain terminating dideoxynucleotide triphosphate and a thermally stable polymerase enzyme (col. 9, line 43, to col. 10, line 45 and col. 13, lines 1-13)." The Examiner further states that Mian et al. differs from the present invention only in that it does not teach a kit. The Examiner further relies on Ahern, which teaches the advantages of a kit.

Applicants respectfully traverse the above rejection on grounds that the Examiner has not demonstrated that the elements of claimed invention are found in either the Mian et al. or Ahern

references. The present invention is directed to a kit containing one or more reaction vessels, each vessel of which contains a mixture of region-specific reagents sufficient to obtain bi-directional sequence (i.e., both the sense and anti-sense strands) of a genomic DNA region of interest. Applicants have amended the claims to clarify that the kit includes "a single reaction vessel for each DNA region to be sequenced containing a mixture of region-specific sequencing reagents sufficient *for sequencing the sense and anti-sense strand of each DNA region to be sequenced.*" The claim language thus requires that each reaction vessel in the kit contain region-specific reagents for obtaining sequence of both the sense and anti-sense strand of the DNA. Mian et al., however, does not teach the above limitations. As highlighted below, Mian et al. teaches a mixture of reagents to obtain sequence for a single nucleic acid strand.

> The method of sequencing a nucleic acid fragment, for example, a DNA fragment, as provided by the invention comprises the following steps. One strand of each of a multiplicity of double-stranded DNA fragments is linked to a tethering molecule and a solution of the multiplicity of tethered double-stranded DNA fragments is placed in the thermomodulating chamber of the meltometer in the presence of a retainer, so that the multiplicity of double-stranded DNA fragments are retained in the thermomodulating chamber. The temperature of the thermomodulating chamber is then raised to a temperature sufficient to thermally denature the multiplicity of DNA fragments. Alternatively, thermal denaturation can be accomplished prior to addition of the DNA sample to the thermomodulating chamber. This thermal denaturation results in there being a multiplicity of single-stranded DNA fragments present in the thermomodulating chamber that are targets for later hybridization with a sequence-specific probe. An oligonucleotide sequencing primer that specifically hybridizes to the DNA fragment at a site in the nucleotide sequence of the DNA fragment adjacent to the site to be sequenced is then annealed to the denatured DNA. This annealing step is performed at a temperature sufficient to allow annealing of the primer to the template DNA to occur. Conventional dideoxynucleotide/replacement synthesis nucleic acid sequencing reactions are then performed to create a nested set of extended oligonucleotides hybridized to the DNA fragment. The temperature in the thermomodulating chamber is then incrementally raised linearly and stringently (.+-.0.01° to 1°C.) at a rate sufficient to detect and resolve denaturation of each of the nested set of extended oligonucleotides hybridized to the DNA fragment of interest. Each species of the nested set of the extended oligonucleotides is then detected by the detector in a temporal sequence that reflects the nucleotide sequence of the DNA fragment. In preferred embodiments, one strand of the multiplicity of DNA fragments, i.e., the strand complimentary to the oligonucleotide primer, is linked to a tethering molecule and retained in the thermomodulating chamber via a retainer. Each of the nested set of extended oligonucleotides is detectably labeled at the 3' terminus by a fluorescently-labeled or an infrared-labeled dideoxy terminator residue. Optionally and advantageously,

> each of the dideoxynucleotides can be differentially labeled so as to be individually detectable by the detector. The thermomodulating chamber is arranged to have a first and second opening whereby buffer flows through the thermomodulating chamber during the course of thermal denaturation. Each of the thermally-denatured, fluorescently- or infrared-labeled extended oligonucleotides then flows past the detector as the result of buffer flow through the thermomodulating chamber and is thereby detected in a temporal sequence that reflects the nucleotide sequence of the DNA fragment.

As noted in the underlined portions above, Mian et al. teaches the use of a single oligonucleotide sequencing primer that hybridizes to a single nucleic acid strand, to produce a "multiplicity of single-stranded DNA fragments" (i.e., a mixture of nested chain-termination DNA fragments). The "mixture" of nested chain-termination fragments is not equivalent to the "mixture" of region-specific sequencing *reagents* sufficient for sequencing the sense and anti-sense strands of each DNA region to be sequenced, as recited in claim 13. Claim 13 has also been amended to clarify that the region-specific sequencing reagents are "region-specific primers." In view of the above differences, Mian et al. does not teach that a single reaction vessel could be used to obtain sequence for both sense and antisense strands of DNA, and does not therefore teach the components of the kit defined in the present claims. The Ahern et al. reference merely discloses the general concept of kits, and does not compensate for the deficiencies of the Mian et al. reference.

Accordingly, neither Mian et al. nor Ahern disclose, alone or in combination, the elements of the claimed invention, and the claimed invention cannot therefore be considered obvious in view of the combined references.

CONCLUSION

In summary, Applicants have amended the claims to more particularly point out and distinctly claim the present invention. The Mian et al. and Ahern references relied upon by the Examiner do not disclose the elements of the claimed invention. Applicants submit that the claims now define subject matter that is neither taught nor suggested by the cited references. For the above reasons, Applicants respectfully request that the claims of the application be allowed and proceed to issuance.

Respectfully submitted,

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